531
IS FUNDAMENTAL THE CROSS TALK BETWEEN ADIPOSE STROMAL CELLS AND OSTEOARTHRITIC CHONDROCYTES OR SYNOVIOCYTES TO MODULATE THEIR BEHAVIOUR?

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Purposes: Adipose stromal cells (ASC) have been shown to exert anti-fibrotic, anti-inflammatory and anti-apoptotic properties, through secreted growth factors, therefore can be good candidates for preventing the evolution of osteoarthritis (OA) disease. Aim of the study was the analysis of trophic potential effects of ASC-conditioned medium (CM) on chondrocyte and synoviocyte from OA patients.
Methods: Good manufacturing practice (GMP)-clinical grade ASC were isolated from subcutaneous adipose tissue. Chondrocytes and synoviocytes were isolated from cartilage and synovial of OA patients undergoing total joint replacement. Chondrocytes or synoviocytes were treated with different ratio of ASC-CM or co-cultured with ASC in transwell. Specific markers of chondrocytes fibrosis (collagen type 1 and 3), hypertrophy (collagen type 10, alkaline phosphatase and MMP-13) and synoviocytes or chondrocytes matrix degrading factors and inhibitors (ADAMTS4, ADAMTS5, TIMP1, TIMP3) were tested by RT-qPCR analysis. Secreted inflammatory (IL6, CXCL8/IL8, CXCL1/GROα, CCL2/MCP-1, CCL3/ MIP1α, CCL5/RANTES) and anabolic (HGF, PGE2) factors by multiplex bead-based sandwich immunoassay or ELISA test.
Results: Chondrocyte treated with ASC-CM significantly inhibited collagen type 1 and 3 and did not affect hypertrophic and matrix degrading factors and inhibitors. By contrast, chondrocytes co-cultured with ASC showed only a reduced expression of both fibrotic and hypertrophyc markers. Synoviocytes treated with ASC-CM decreased ADAMTS5 and increased ADAMTS4 expression while no effects were observed on TIMP1 and TIMP3. By contrast, synoviocytes in co-culture experiments confirmed a decrease of ADAMTS5 associate to an increase of TIMP1expression. The release of all the inflammatory factors analyzed were decreased in chondrocytes and synoviocytes co-culture experiments while ASC-CM reduced only the expression of CXCL8/IL8 and CXCL1/GROα in chondrocytes. HGF was significantly induced treating chondrocytes with both ASC-CM or ASC in co-culture. PGE2 was mainly induced only in co-culture experiments.
Conclusion: These data demonstrate that all the markers analyzed on chondrocytes or synoviocytes were mainly down-modulated in co-culture condition suggesting the importance of the cross-talk between cells and supporting the importance of using ASC in the treatment of OA. Moreover, even if HGF and PGE2 were involved in down-modulation of fibrosis and inflammation, however, ASC effects were strictly dependent from their presence indicating that ASC-CM could be less efficacious in preventing OA evolution.

532
ELEVATED SYNOVIAL FLUID CONCENTRATION OF ADENOSINE TRIPHOSPHATE IN DOGS WITH OSTEOARTHRITIS OR SODIUM URATE-INDUCED SYNOVITIS/INFLAMMATION OF THE STIFLE

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Purpose: Currently, the association between osteoarthritis (OA) and joint pain is unpredictable and is not linear. Both objective and subjective outcome measures have been used with varying degrees of correlation. Establishing an outcome measure that could be correlated with a decrease in the actual joint pain pathway could prove beneficial to researchers and clinicians in accurately assessing the therapeutic efficacy, especially if it can be correlated to either of the current indirect outcome measurement systems used. Studies of OA pain involving biomarkers are limited. One study demonstrated a correlation between synovial fluid (SF) adenosine triphosphate (ATP) concentrations and OA knee pain in humans. It has been documented that ATP is secreted by chondrocytes secondary to mechanical stress. In peripheral tissues, ATP mediates nociception, mechanosensitivity, and thermal sensitivity, and central blockade of ATP receptors has been shown to constitute a key step in chemosensory transduction. Currently, there are no reports describing the presence or role of SF ATP concentrations in naturally occurring canine OA or in a urate-induced synovitis (UIS) model of inflammation. Our hypothesis is that elevated SF ATP concentrations are present in the stifle joints of dogs with either naturally occurring OA or UIS as compared to the SF in normal control stifle joints.
Methods: SF was collected from 3 groups of dogs. SF was collected unilaterally from the stifle joints of 26 normal dogs; 25 dogs with sodium urate-induced synovitis (UIS) of the stifle joint; and 32 client owned with naturally occurring stifle joint osteoarthritis (OA) as determined from stifle radiographs and physical examination. Experimentally induced synovitis/inflammation was performed with a UIS model. The dogs with OA had SF obtained while under general anesthesia prior to cruciate reconstruction surgery. Cruciate ligament status (partial vs. complete tear) and meniscal pathology (intact vs. torn) were recorded. Normal and UIS dogs were premedicated with butorphanol (0.3mg/kg) intramuscularly and propofol (4mg/kg) was given intravenously prior to SF collection. For all dogs, a standard stifle joint arthrocentesis was performed. Only clear, yellowish samples were used for determination of ATP concentration. Hematologic and serum biochemical profiles and complete physical examinations were performed prior to fluid collections. ATP concentration was determined with a bioluminescence assay utilizing recombinant firefly luciferase and D-luciferin. Radiographic assessment of the stifle was performed in the OA dogs and evaluated by a radiologist and scored using an established scoring system consisting of 16 individual determinants (Table 1). Comparison of SF from the 3 groups was performed with the Kruskal-Wallis test and multiple comparisons were performed with Dunn's test. All hypothesis tests were 2-sided with significance at α=0.05 (Prism, v6.0). Evaluation of the effect of cruciate or meniscal pathology, and radiographic score on SF ATP levels in OA dogs was performed with multivariate linear regression analysis with significance at α=0.05 (Stata/SE, 13.0).
Results: SF ATP from normal dogs was significantly lower than the SF from UIS ($p<0.001$) or OA ($p<0.001$). There was no difference between UIS and OA ($p=0.639$) (Figure 1). Cruciate ligament status ($p=0.218$), meniscal status ($p=0.308$), and total radiographic score ($p=0.826$) were not predictive of SF ATP concentration in the OA group. However, when individual determinants of the radiographic score were assessed, the presence of osteophytes in the femoral intercondylar notch (#8; Table 1) was significantly correlated with SF ATP ($p=0.007$).

Table 1
Subjective Scoring System for Radiographic Evaluation

Individual Determinants
1. Patellar osteophytes
2. Femoral trochlear groove periarticular osteophytes
3. Lateral and/or medial femoral condylar periarticular osteophytes
4. Femoral subchondral sclerosis
5. Distal femoral condylar remodeling
6. Subchondral cystic lucencies
7. Sesamoid periarticular osteophytes
8. Femoral intercondylar notch osteophytes
9. Proximal tibial periarticular osteophytes
10. Proximal tibial subchondral sclerosis
11. Proximal tibial subchondral cystic lesions
12. Central tibial plateau osteophytes
13. Joint effusion/capsular thickening
14. Intraarticular mineralized osseous fragments
15. Meniscal mineralization
16. Intercondylar avulsion fracture fragments

*Determinants are assigned a value of 0=absent; l=mild; 2=moderate; 3=severe
*The sum of all individual radiographic determinants produces the total radiographic score for an individual stifle joint